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TITLE: NONINVASIVE MONITORING OF TISSUE OXYGENATION AND REDOX STATUS IN HUMANS

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13. ABSTRACT (Maximum 200 words)

This report results from a contract tasking St. George's Medical School to investigate a cluster of reliable noninvasive techniques for the tracking of improvements in oxygen delivery, oxygen availability and oxygen usage within and in the vicinity of sites of injury. The investigator evaluated a range of state-of-the-art commercially available techniques designed to monitor the status of tissue hemodynamics and energetics in humans, both within and upstream of selected locations, such as the limbs and the brain. The techniques included near infrared spectroscopy and Doppler based ultrasound monitoring of regional blood velocities and flows.

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1. INTRODUCTION

Disorders of local metabolism and/or blood flow in the extremities occur in a range of conditions. The primary objective of this investigation was to identify a cluster of techniques which have the potential for reliable, noninvasive tracking of the degree of impairment of tissue O_2 delivery and O_2 usage, and improvements (if any), which may be induced.

Evaluations were therefore undertaken of (i) near infrared spectroscopic (NIRS) monitoring of local tissue oxygenation (including redox status) and (ii) Duplex Doppler ultrasound monitoring of regional blood flow to muscle groups in the legs, both in normal healthy subjects and in patients with claudicating peripheral vascular occlusive disease. These measurements were made under conditions designed to challenge muscle tissue oxygenation status by manipulation of (i) metabolic rate, (ii) vascular perfusion and (iii) arterial blood oxygenation.

2. EXPERIMENTAL DESIGN AND PROCEDURES

2.1 SUBJECTS

Following approval by the Institutional Ethics Committee, a group of 18 normal healthy subjects participated in the investigation. In addition, further smaller group of 6 patients with symptoms of claudicating peripheral vascular occlusive disease were studied; some of these served as their own controls, i.e. one leg was predisposed to claudication while the other was uninvolved.

2.2 METHODS

Subjects breathed through a mouthpiece connected to a turbine monitor for measurement of inspiratory and expiratory volume. Respired PCO_2, PO_2 and PN_2 were determined by mass spectrometry from a sample drawn from the mouthpiece. Heart rate was derived from the R-R interval of the ECG signal. Arterial O_2 saturation was monitored noninvasively by pulse oximetry. The electrical signals from these devices underwent analog-to-digital conversion and computer analysis for on-line, breath-to-breath determination of pulmonary gas exchange and ventilatory variables.

2.2.1 Exercise

Several exercise modes were undertaken:

a. Cycle ergometer exercise: An electromagnetically-braked, computer-controlled cycle ergometer (Lode) was used.

b. "Isolated" rhythmic exercise of the quadriceps: This utilized rubber bands of length and recoil characteristics to deliver the required increment of metabolic

rate and limb blood flow. These were arranged with stirrups placed over the dorsal surface of the foot for the alternate quadriceps contractions, with wooden stops providing the required excursion (~75^{o} from the vertical), and a metronome setting the cadence. Following the active contraction phase, the return to the initial position was achieved chiefly through the device recoil rather than by contraction of the antagonist muscles, thereby "isolating" the work to the quadriceps muscles.

c. "Isolated" rhythmic exercise of gastrocnemius-soleus muscles: The exercise comprised the rhythmic alternate plantar flexion of the foot, against the resistance resulting from a variably-weighted pulley system. Wooden stops set the excursion, and a metronome set the cadence.

d. Maximal voluntary contractions (MVC): Isometric contractions of the quadriceps muscles (at a knee angle of 90^{o}) were performed to the limit of tolerance.

Subjects completed a range of exercise protocols:

a. Incremental (ramp) exercise: This test was undertaken on the cycyel ergometer to the subjects' limit of tolerance, using work-rate incrementation rates designed to lead to fatigue within ~10-12 min (eg. 15 Watts.min^{-1}). In addition to the recording of pertinent physiological responses over the entire range of exercise intensities, this test also allowed the maximal O_2 uptake ($\dot{V}O_2max$) to be determined and the lactate threshold (θ_L) to be estimated for the exercise mode under investigation, using non-invasive pulmonary gas exchange criteria (Whipp et al, 1986).

b. Moderate-intensity square-wave exercise: Square-wave tests were performed from a baseline of rest or light exercise. Work rates, which were constrained to lie below θ_L (eg. 80 Watts on the cycle ergometer for the normal subjects), were imposed for 6-8 min.

c. High-intensity square-wave exercise: Square-wave tests were performed as described above, but to work rates that were supra-θ_L and which were performed to the limit of tolerance.

2.2.2 Alterations in Arterial Oxygenation

Tissue hypoxia was simulated by means of acute arterial hypoxemia. This was induced by causing subjects to breathe 12% O_2 (at normal atmospheric pressure) which typically lowered alveolar (end-tidal) PO_2 to ~50-55 mm Hg, for periods no longer than 10 min. To induce acute arterial hyperoxemia, subjects breathed 100% O_2, again for no longer than 10 min, increasing alveolar PO_2 to over 600 mm Hg.

2.2.3 Vascular Occlusion

Reductions in perfusion to the leg musculature were accomplished by the inflation of a pneumatic cuff at the level of the thighs (ie. to restrict arterial vascular inflow and venous outflow).

2.3 NIR SPECTROSCOPY

The response profiles of muscle tissue O_2 supply, O_2 utilization, redox status and local blood volume change were derived by NIRS (NIRO 500, Hamamatsu, Japan), using wavelengths appropriate for the various chromophores. Temporal profiles of change in the following variables were obtained: [oxyhemoglobin] (HbO_2), [deoxyhemoglobin] (Hb), oxidized [cytochrome aa3] (CtO_2) - the terminal enzyme of the mitochondrial electron transport chain, and local blood volume (Vb) (assumed to be proportional to the change in total hemoglobin). The transmitting and receiving optodes were placed 4 cm apart on the thigh over the vastus lateralis, and taped securely in place (to prevent motion) and covered with a light-excluding bandage (to minimise interference from external light sources and the escape of light from the laser source).

2.4 DOPPLER ULTRASOUND

Blood velocity and vessel cross-sectional area were monitored continuously from the common femoral artery (CFA) and its major branches - the superior femoral and profunda femoris arteries (SFA, PFA) in the inguinal region of the thigh. Blood flow was derived beat-by-beat, using colour Duplex ultrasound (ATL Ultramark 9, Advanced Technology Laboratories, Seattle, USA) (Fig 1). This device combines an imaging system (real-time B-mode ultrasonic scanner) with a Doppler system (single gate Pulsed Doppler unit). The B-mode scanner uses echo-ultrasonography to provide real-time two-diemnsional monochrome images of the vessel, allowing the Doppler gate (the area from which the Doppler shift frequencies are sampled) to be positioned correctly (ie. encompassing the entire vessel). The spatial mean velocity (ie. the mean of all the velocities over the vessel cross-section) was calculated instantaneously from the spectrum of Doppler shift frequencies, and averaged over 5 cardiac cycles. Vessel diameter was measured from a B-scan picture taken at systole; the vessel was assumed to be circular in cross-section, with its diameter remaining constant throughout the measurement period.

3. RESULTS AND DISCUSSION

3.1 ALTERED METABOLIC RATE

It proved possible to reliably discriminate dynamic response profiles for both vascular perfusion and muscle oxygenation status during the various exercise protocols, spanning an oxygen uptake ($\dot{V}O_2$) range of ~ 0.75 to 4.2 l/min.

3.1.1 Doppler monitoring of vascular perfusion

The advantage of Doppler-based techniques over conventional techniques for monitoring of vascular perfusion (eg. venous occlusion plethysmography, indicator dilution, thermodilution) is that changing responses can be followed. While early Doppler-based approaches are now relatively common in clinical monitoring, they commonly rely on the measurement of blood velocity, with the subsequent derivation of $\dot{Q}$ depending on the questionable assumptions that both the cross-sectional area and the angle of insonation of the vessel at the point of measurement remain constant.

We overcame this major technical and intrepretational limitation by employing the relatively new technique of Duplex Doppler ultrasound. This confers the advantage of measuring both blood velocity and vessel diameter, therefore allowing $\dot{Q}$ to be calculated. The calculated time-averaged mean vessel velocity has been argued to be independent of the $\dot{Q}$ profile (ie. laminar, transitional, turbulent) (Evans et al 1989). The ability to monitor flow by this technique should therefore be valid for larger vessels and branching configurations, as well as smaller vessels, as long as the vessel is accessible. However, as the technique is susceptible to noise from any motion of the vessel under study, it is important that the probe be maintained stationary with respect to the vessel.

The Duplex Doppler technique allowed us to successfully monitor blood flow not only in a major systemic artery - the common femoral artery - but also in its smaller subdivisions - the superior femoral and profunda femoris arteries (SFA, PFA) (Fig 2). One of the major difficulties encountered in this phase of the investigation was the presence of movement artefacts which affected the ability to maintain a stable position for the monitoring probe over the vessel in question. Thus, while it proved possible to monitor CFA flow ($\dot{Q}_{CFA}$) in the recovery phase from cycle-ergometer exercise of various intensities (ie. $\dot{V}O_2$ averaging 1.3 and 1.7 l/min for the light and moderate intensities, respectively) (Fig 2), the on-transients were contaminated with noise to an extent that rendered them of poor analytical value. However, when the inguinal region was stabilized - as the localized quadriceps format allowed - clear and reproducible $\dot{Q}_{CFA}$ profiles were obtained both for the exercise and recovery phases. A typical $\dot{Q}_{CFA}$ and $\dot{V}O_2$ response to a square-wave of moderate-intensity quadriceps exercise is shown in Fig 3 for a normal subject.

The on-transient time constant (τ) of $\dot{Q}_{CFA}$ (28.8 ± 4.4 s; mean ± SEM) was faster than $\tau\dot{V}O_2$ (41.5 ± 7.2 s), such that the ratio $\tau\dot{V}O_2$:$\tau\dot{Q}_{CFA}$ was 1.49 ± 0.12 (Fig 4). There was no significant difference in $\tau\dot{V}O_2$ between the on- and the off-transient (38.0 ± 5.5 s). $\tau\dot{Q}_{CFA}$, however, was significantly slower at the off-transient (40.8 ± 3.8 s) than at the on-transient (Figs 3 & 4). These results suggest that, in normal subjects for moderate metabolic stress: (a) the ability of the tissue to utilize oxygen is dictated by processes within the tissue itself rather than by the ability of the perfusion to deliver oxygen to the tissue (ie. the limb blood flow response develops significantly more rapidly than does

$\dot{V}O_2$); and (b) the slower $\dot{Q}_{CFA}$ response as metabolic rate fell serves an important control role, which reduces the potential for tissue hypoxia.

$\dot{Q}_{CFA}$ measurements were also carried out on patients with peripheral vascular occlusive disease. Square-wave transitions were performed from rest, using plantar flexion (Fig 5). These work rates were smaller than those imposed with quadriceps exercise, because of the smaller muscle mass (ie. $\dot{V}O_2$s typically between 0.6 and 0.8 l/min).

The ability to monitor blood flow in small vessels is also illustrated by the responses shown in Fig 2. This shows the recovery profiles from a short bout of "all-out" cycle-ergometer exercise in the CFA and in its smaller subdivisions, the SFA and PFA. We demonstrated that flow profiles during rhythmic plantar flexion of the foot could be successfully monitored in small arteries of the foot. The dynamics of the flow responses were clearly abnormal in the subjects with peripheral vascular occlusive disease, reflecting either an increased time constant (Fig 5, middle panel) or smaller response gain (Fig 5, lower panel).

These experiments demonstrate that Duplex Doppler techniques can be used to monitor dynamic response profiles of arterial perfusion in response to abruptly imposed increases and decreases in muscle tissue metabolic rate. It is important, however, to recognize that there are potential sources of error associated with this technique. For example:

(a) There is a lack of suitable, commercially-available flow phantoms with which to perform dynamic calibration over a wide range of flows.

(b) If the sample volume from which Doppler signals are being received is smaller than the blood vessel in which velocity is being measured (incomplete insonation), the spatial mean velocity that is calculated will not take account of the full velocity profile in the vessel cross-section. And

(c) Vessel cross-sectional area is continuously varying as consecutive pulse waves travel down the length of the vessel, an effect which is likely to be more marked in smaller vessels because of their greater compliance. It is important, therefore, that measurements are taken at the same point in the cardiac cycle which, by convention, is at systole.

In vitro and in vivo studies (Gill, 1985) indicate a random error of the order of 10-15% associated with the technique.

3.1.2 NIR spectroscopy

Most of the investigations utilizing NIRS in humans have been performed on the brain (eg. Jobsis, 1977; Brazy et al, 1985; Brazy & Lewis, 1986; Elwell et al, 1992). Monitoring skeletal muscle is more problematic: motion of the muscle during exercise make it difficult to stabilize the optode assembly on the limb surface; there is uncertainty

regarding the most appropriate path length for optimizing light transmission and collection through the muscle tissue rather than subcutaneous fat and bone; and the extent to which the path length is affected during exercise is unknown.

In response to exhausting ramp incremental exercise on a cycle ergometer, there was a progressive marked hemoglobin desaturation (ie. increased [Hb]), consistent with an increased tissue arterio-venous O_2 difference (Fig 6). However, despite evidence of a metabolic (lactic) acidosis at higher work rates, CtO_2 was not systematically reduced, even in those subjects who evidenced marked cytochrome aa3 reduction in response to a maximum isometric contraction (Fig 6). We show one instance of a small [CtO_2] reduction during the ramp exercise, but note that there was apparently a further reduction during recovery, despite improved blood Hb oxygenation. The results suggest that either the metabolic acidosis of high-intensity muscular exercise is not a result of O_2 limitation at its utilization site at cytochrome aa3, or that the O_2-limited sites are proportionally too small to be discriminated by this regional-average technique.

For moderate and heavy cycle-ergometer exercise, the kinetics for $\dot{V}O_2$ and quadriceps O_2 extraction (ie. increasing [Hb]) were similar (Fig 7). Further analysis showed that the rate of hemoglobin desaturation in the exercising muscle was proportional to the rate of $\dot{V}O_2$ for all the imposed work rates.

Changes in muscle blood volume were clearly discernible from the change in total hemoglobin concentration (ie. the sum of reduced and oxygenated hemoglobin concentration changes). For example, exercise hyperemia was evident for moderate and heavy square-wave cycle-ergometer exercise, ramp incremental cycle-ergometer exercise, and graded and maximal voluntary contractions of the quadriceps. Post-exercise hyperemia was also evident, especially at high work rates.

One area of uncertainty was the physiological significance of CtO_2 changes. As discussed earlier, no systematic or reproducible evidence was found of an increased reduction of CtO_2 at maximal exercise on the cycle ergometer. With maximal voluntary contractions of the quadriceps, a range of CtO_2 profiles was seen: no change, increase or decrease. What was disconcerting, however, was that a particular subject could manifest any of these responses. This lack of consistency leads us to conclude that NIRS is not at present a sufficiently robust technique for monitoring muscle tissue redox status (ie. the actual concentration changes in cytochrome aa3 are extremely small).

This concern apart, while NIRS was successful in tracking dynamic changes in muscle tissue oxygenation in response to small and large changes in metabolic rate, there are nonetheless some limitations to the technique. For example, responses cannot yet be adequately quantitated as absolute concentrations, only as concentration changes. Also, reliable estimates of path length for muscle are not yet available.

3.2 ALTERATIONS IN ARTERIAL OXYGENATION

NIRS was used to track changes accruing from hypoxic and hyperoxic challenges both at rest and during exercise. Following the abrupt switching between these inspirates, small but consistent changes were evident in Hb oxygenation (eg. Fig 8). No systematic changes in [CtO2] were discernible, however.

3.3 ALTERATIONS IN TISSUE PERFUSION

NIRS was used to monitor the consequences for muscle tissue oxygenation with reductions in perfusion to the quadriceps. This was accomplished by (i) the progressive inflation of pneumatic cuffs at the level of the thighs (ie. to restrict arterial vascular inflow and, even more, its venous outflow) and (ii) sustained isometric contractions of the involved musculature (Fig 6). In both instances, a clear and progessive desaturation was evident (ie. [Hb] increasing with time). Again, however, no systematic changes in [CtO2] could be discerned.

4. SUMMARY

The results of these investigations provide, we believe, evidence for the utility of measuring the dynamics of tissue flow and/or oxygenation using recently developed techniques. However, future studies will be needed to provide information on the quantitative sensitivity of these techniques; ie. their ability to monitor small changes in O2 delivery and utilization in tissues of particular interest under conditions such as HBO therapy not only the limbs, but also the brain. The results appear to be extremely promising.

A further phase of investigation is therefore recommended for this purpose, and also for a clinical phase of investigation to address the effects of altered tissue O2 delivery and utilization in both healthy subjects and also in patients with a range of conditions leading to impaired regional vascular O2 delivery.

5. REFERENCES

Brazy, JE, DV Lewis, MH Mittnickc, and FF Jobsis. Noninvasive monitoring of cerebral oxygenation in preterm infants: preliminary observations. Pediatrics 75:217-225, 1985.

Brazy, JE, and DV Lewis. Changes in cerebral blood volume and cytochrome aa3 during hypertensive peaks in preterm infants. Pediatrics 108:983-987, 1986.

Elwell, CE, M Cope, AD Edwards, JS Wyatt, DT Delpy, and EOR Reynolds. Measurement of cerebral blood flow in adult humans using near infrared spectroscopy - methodology and possible errors. Adv Exp Med & Biol, 1992.

Evans, DH, WN McDiken, R Skidmore, and JP Woodcock. Doppler Ultrasound. Chichester: Wiley, 1989.

Gill, RW. Measurement of blood flow by ultrasound: accuracy and sources of error. Ultrasound Med Biol 11:625-641, 1985.

Jobsis, FF. Noninvasive, infrared monitoring of human cerebral and myocardial oxygen sufficiency and circulatory parameters. Science 198:1264-1267, 1977.

Whipp, B.J., S.A. Ward, and K. Wasserman. Respiratory markers of the anaerobic threshold. Adv. Cardiol. 35:47-64, 1986.

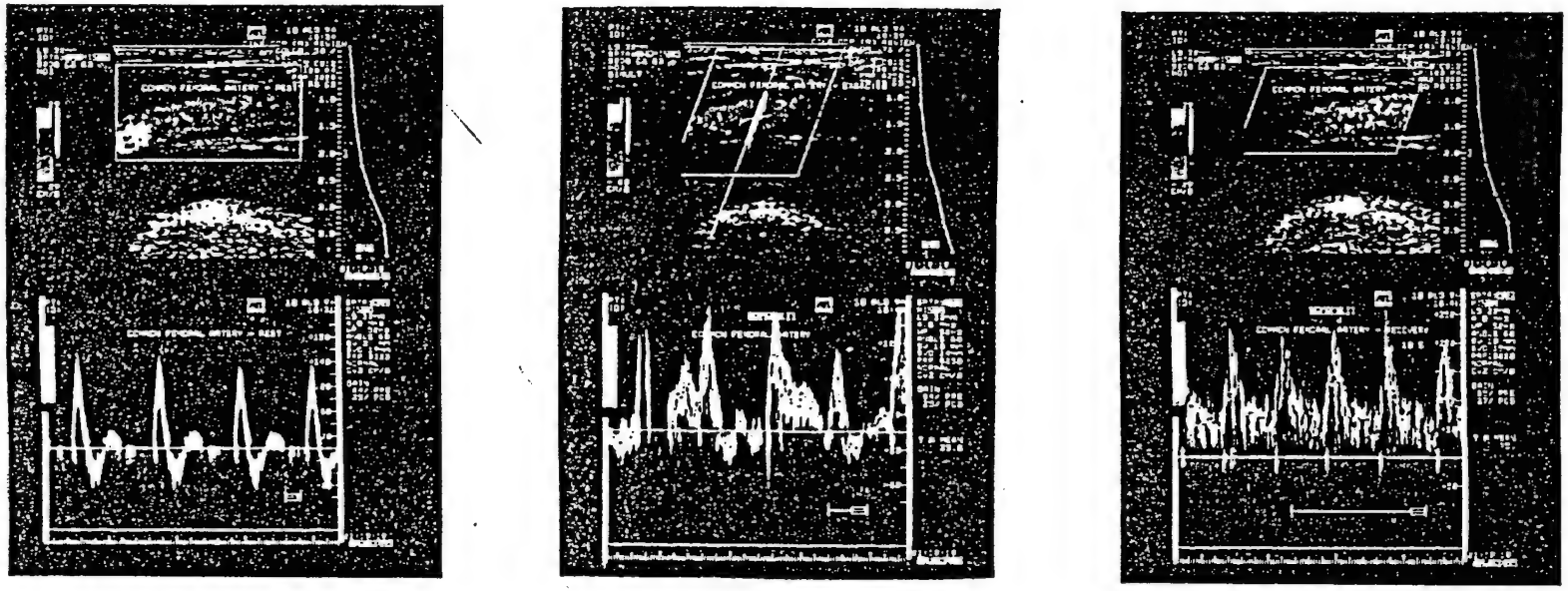

Figure 1. Blood flow velocity profiles and vessel dimensions of the common femoral artery for a normal subject at rest (*left*), during rhythmic quadriceps extensions (*centre*) and during recovery (*right*). Note that the oscillations during the excercise reflect both cardiac and skeletal muscle contractions. The white bar is an example of the Doppler "gate".

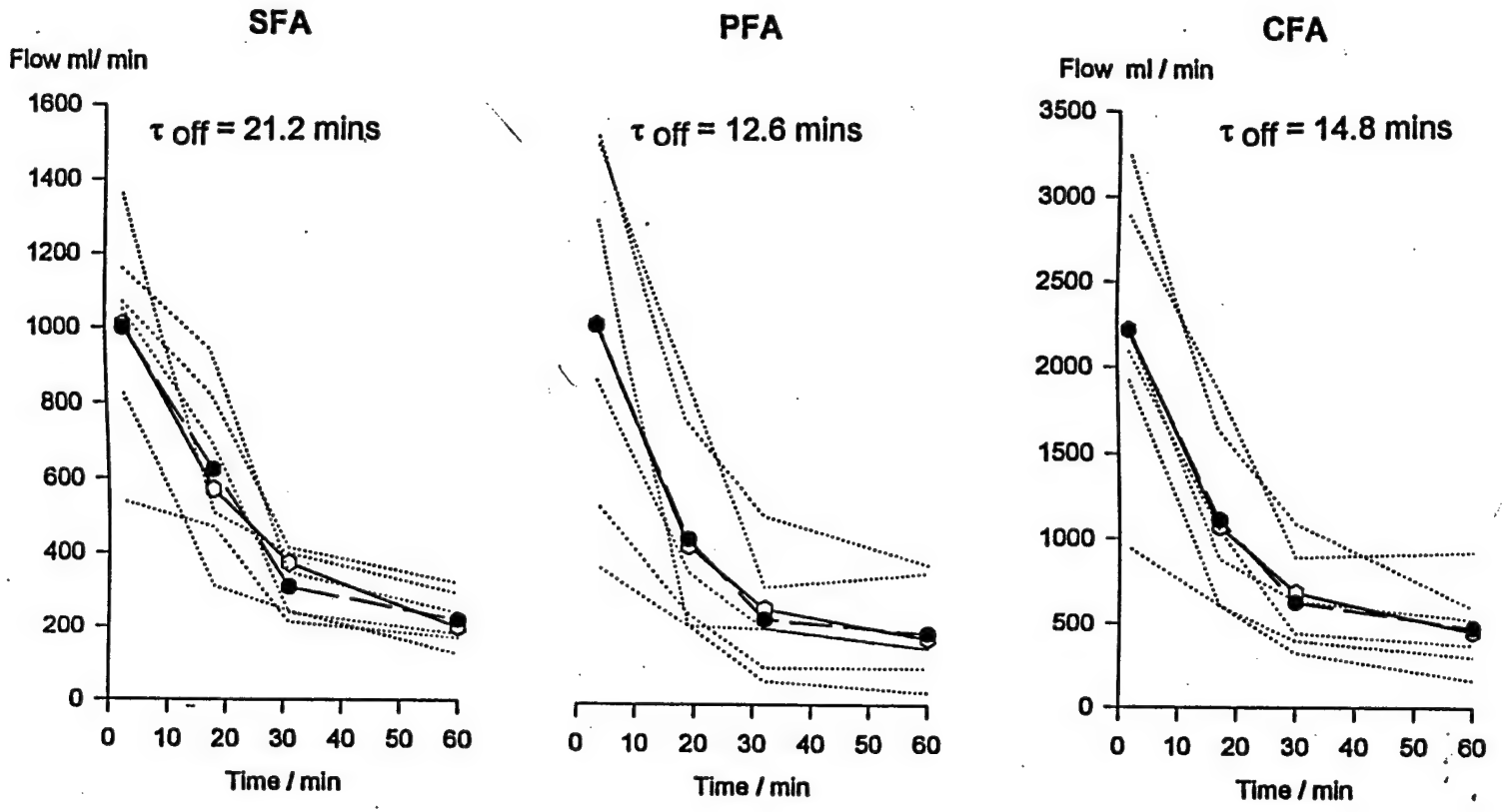

Figure 2. Profiles of blood flow in the profunda femoris (PFA), superior femoral (SFA) and common femoral (CFA) arteries at the off-transient of exhausting high-intensity cycle-ergometer exercise. Curve fits (*thick dashed lines*) were applied to mean response profiles (*thick solid lines*); individual response profiles are shown by thin dashed lines.

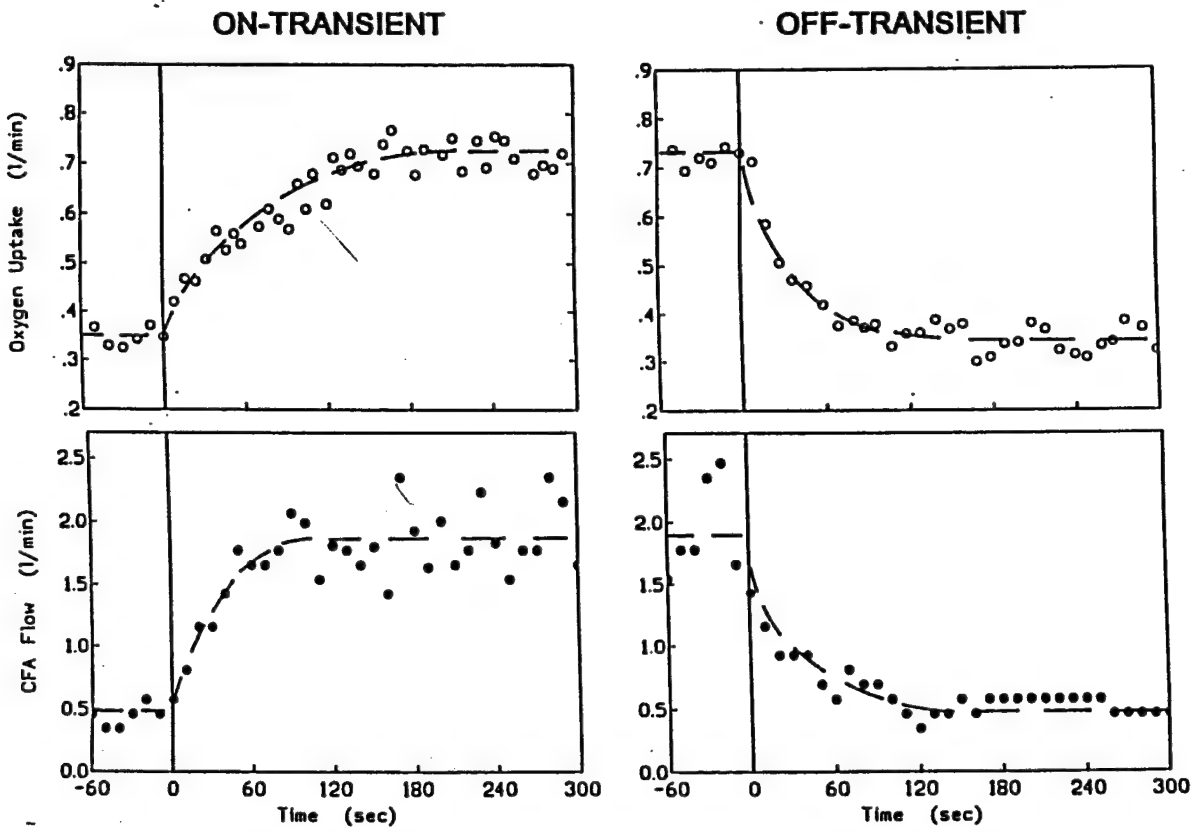

Figure 3. Dynamic response profiles of O2 uptake (l/min) and common femoral artery flow (l/min) between rest and the steady state of rhythmic quadriceps extensions (*left*) and the subsequent recovery (*right*). Note that the dynamics are clearly discernible with our exercise protocol, and that they are faster for flow than for O2 uptake.

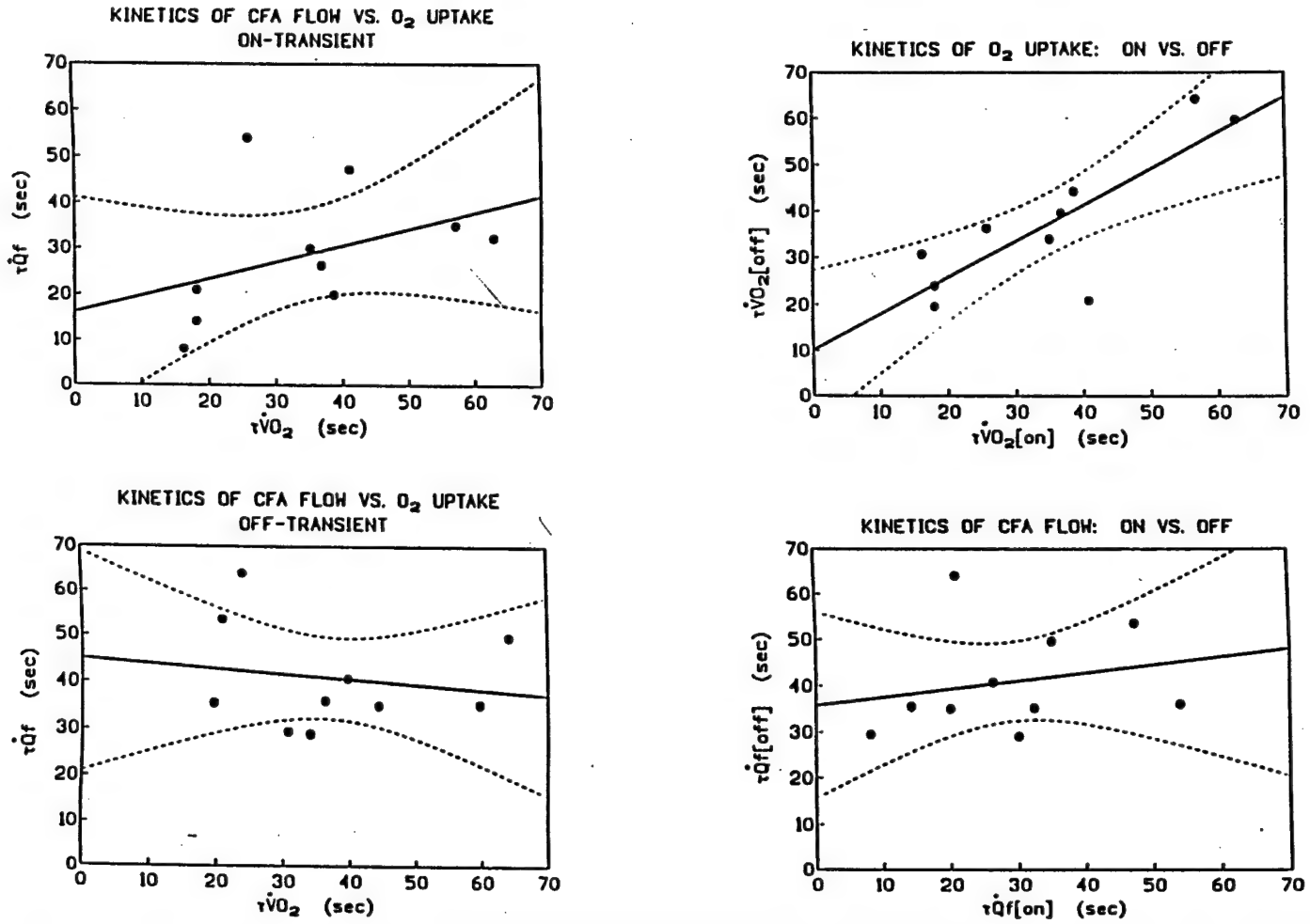

Figure 4. *Left.* Relationship between the time constant for common femoral artery flow ($\tau\dot{Q}f$) and O2 uptake ($\tau\dot{V}O2$) for the on-transient of rhythmic quadriceps extensions (*above*) and the off-transient (*below*). *Right.* Relationship between $\tau\dot{V}O2$ for the on-transient aand the off-transient (*above*), and between ($\tau\dot{Q}f$) for the on-transient and the off-transient (*below*).

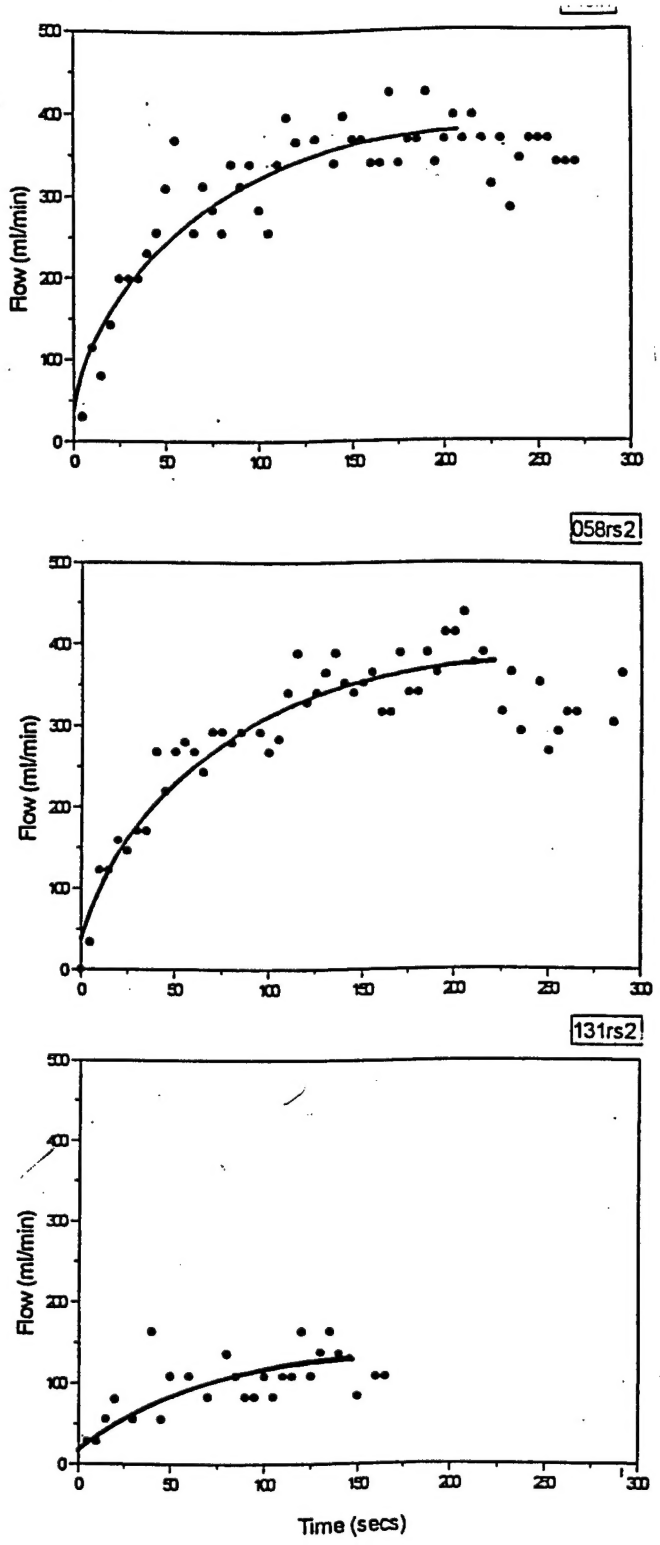

Figure 5. Dynamic response profile of common femoral artery flow ($\dot{Q}f$) between rest and the steady state of rhythmic plantar flexions for a healthy subject (*upper*) and two patients with peripheral vascular occlusive disease (*middle*, *lower*).

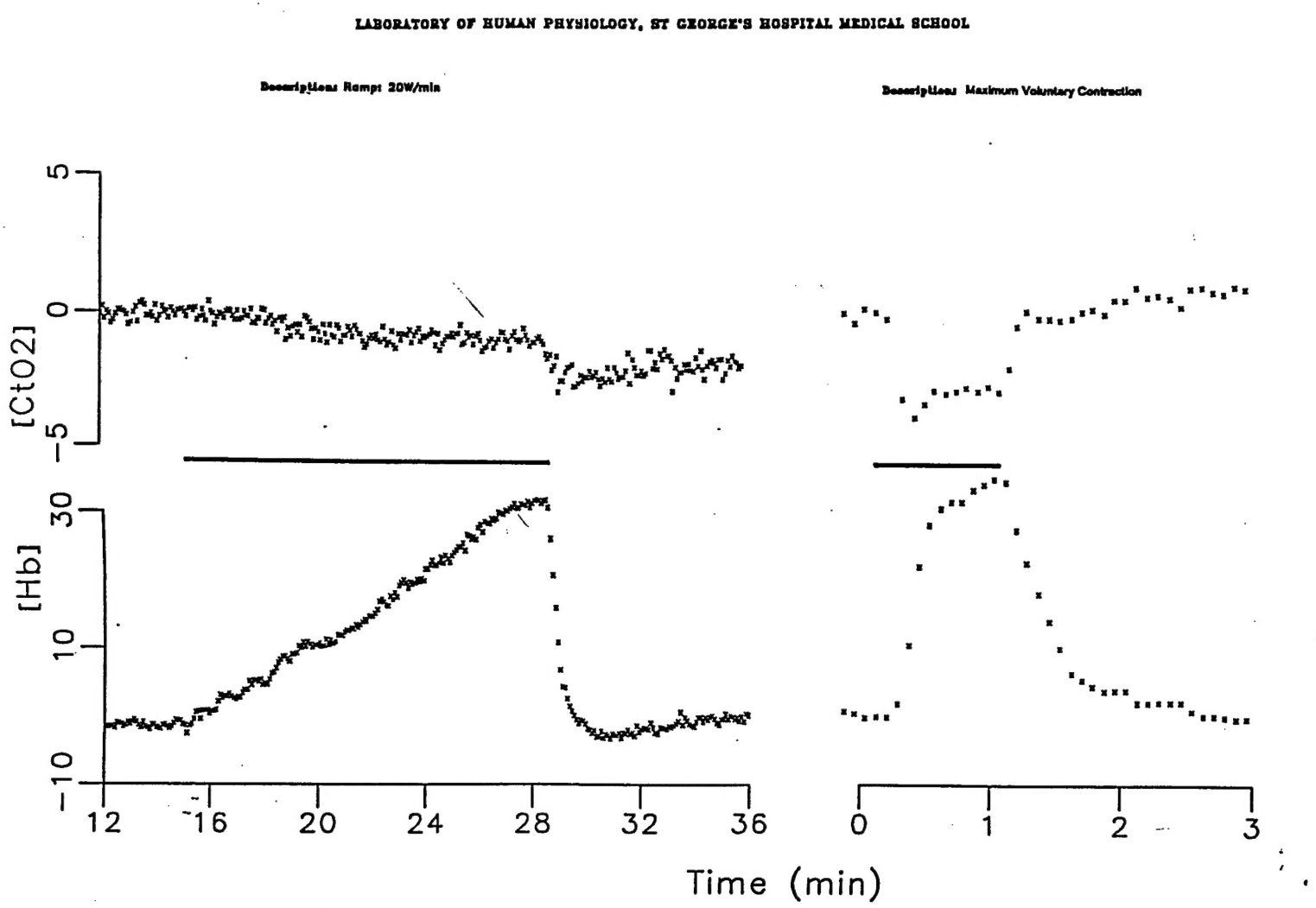

Figure 6. Concentration profiles of deoxygenated hemoglobin [Hb, μM] (*below*) and oxidised cytochrome aa3 [CtO2, μM] (*above*) in the dominant quadriceps femoris muscle during an exhausting ramp test on a cycle ergometer (*left*) and a maximum voluntary contraction (*right*). Solid bars indicate duration of the exercise maneuvers. Concentrations are presented as changes from control (ie. 0).

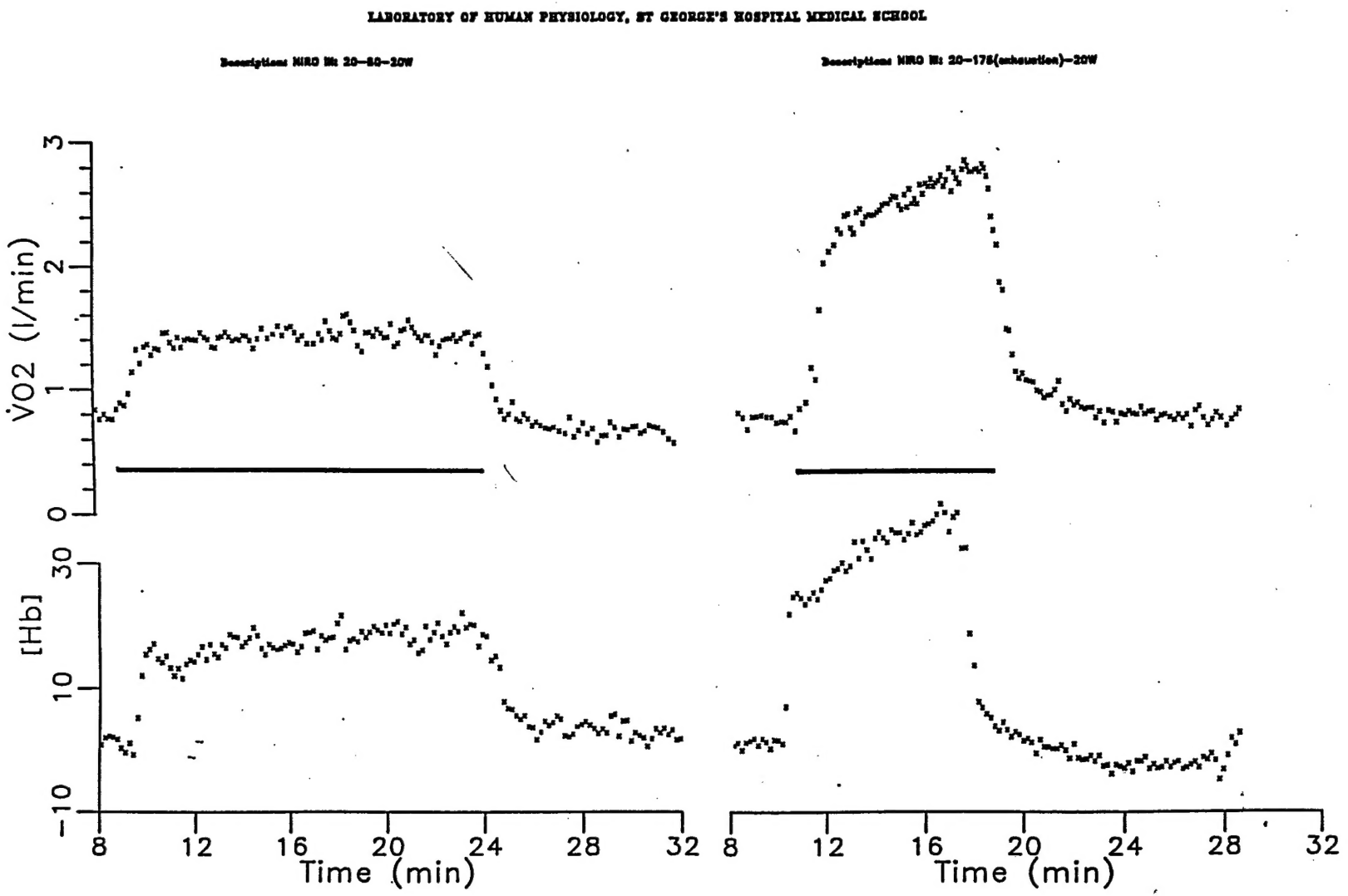

Figure 7. Concentration profiles of deoxygenated hemoglobin [Hb, μM] (*below*) and O2 uptake ($\dot{V}O_2$) (*above*) in the dominant quadriceps femoris muscle during square-wave exercise on a cycle ergometer performed below the lactate threshold (*left*) and above the lactate threshold to

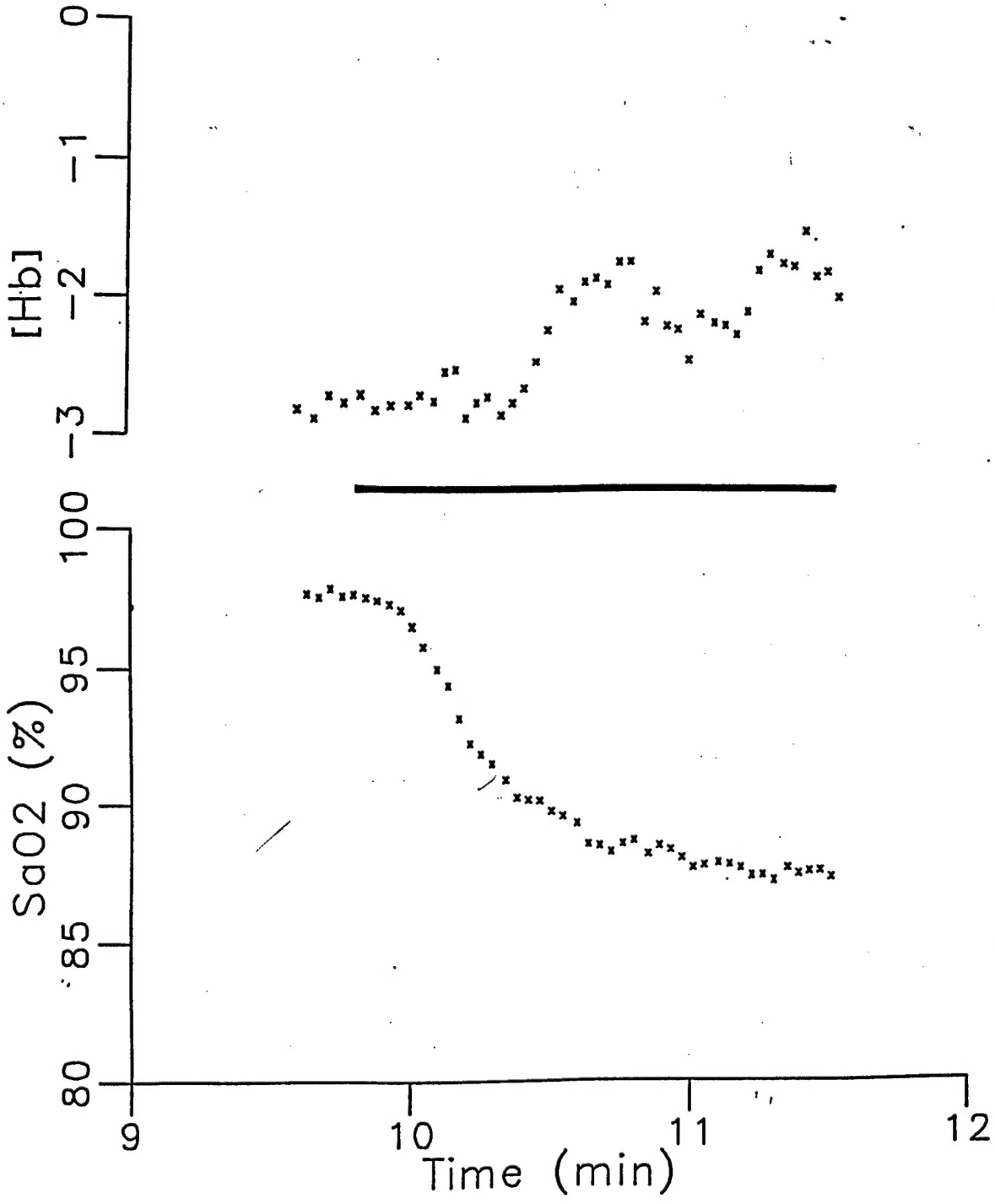

Figure 8. Concentration profiles of arterial oxyhemoglobin (SaO_2) (*below*) and deoxygenated hemoglobin [Hb, μM] (*above*) in the dominant quadriceps femoris muscle on a cycle ergometer